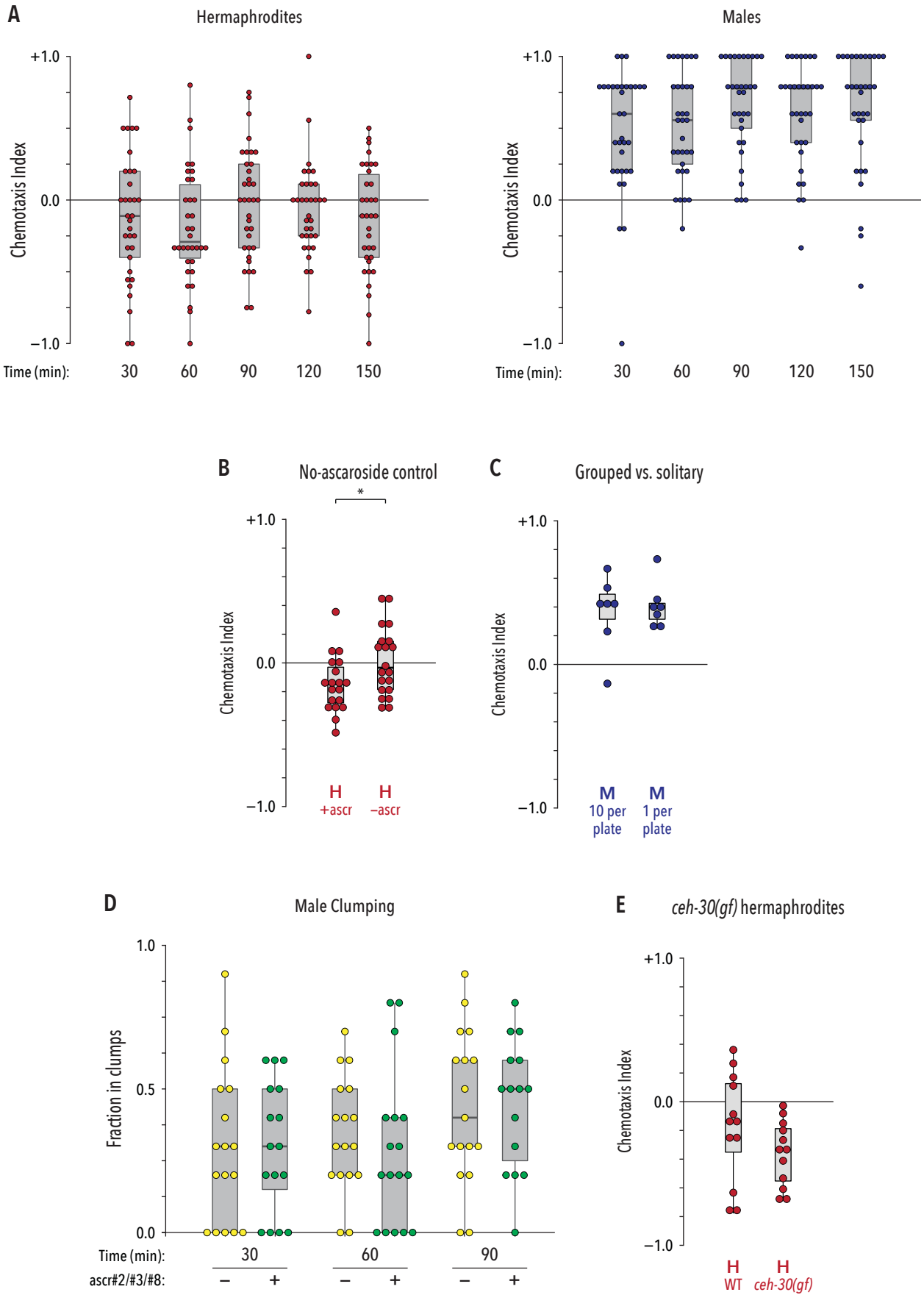
A
Hermaphrodites
Males
Chemotaxis Index
+1.0
0.0
−1.0
Time (min):
30
60
90
120
150
B
No-ascaroside control
*
H
+ascr
H
−ascr
C
Grouped vs. solitary
M
10 per plate
M
1 per plate
D
Male Clumping
Fraction in clumps
1.0
0.5
0.0
Time (min):
ascr#2/#3/#8:
−
+
E
ceh-30(gf) hermaphrodites
H
WT
H
ceh-30(gf)

Figure S1. Behavioral responses to ascarosides, related to STAR Methods (Method Details, Behavioral Assays) and Figure 1.

(A) Chemotaxis Indices for wild-type animals at 30-min intervals. Over the course of 150 minutes, there was no evidence of adaptation to the ascaroside stimulus. (B) Hermaphrodite behavior in the standard quadrant assay ("H +ascr") was significantly different from behavior in a mock experiment using plates with four control quadrants ("H –ascr"), demonstrating that hermaphrodites are indeed weakly repelled from the ascaroside mixture used here. (C) Males tested singly in the quadrant assay ("1 per plate") behaved similarly to those tested in groups of ten. For the "1 per plate" data, the positions of ten singly assayed animals were pooled to obtain one Chemotaxis Index value. (D) The presence of ascarosides had no detectable effect on the propensity of males to form clumps. (E) The *ceh-30(gf)* mutation, which causes hermaphrodites to retain CEM neurons, had no detectable effect on hermaphrodite behavior in the quadrant assay. In (B-E), pairs of groups were analyzed using Mann-Whitney tests. *$p \leq 0.05$.

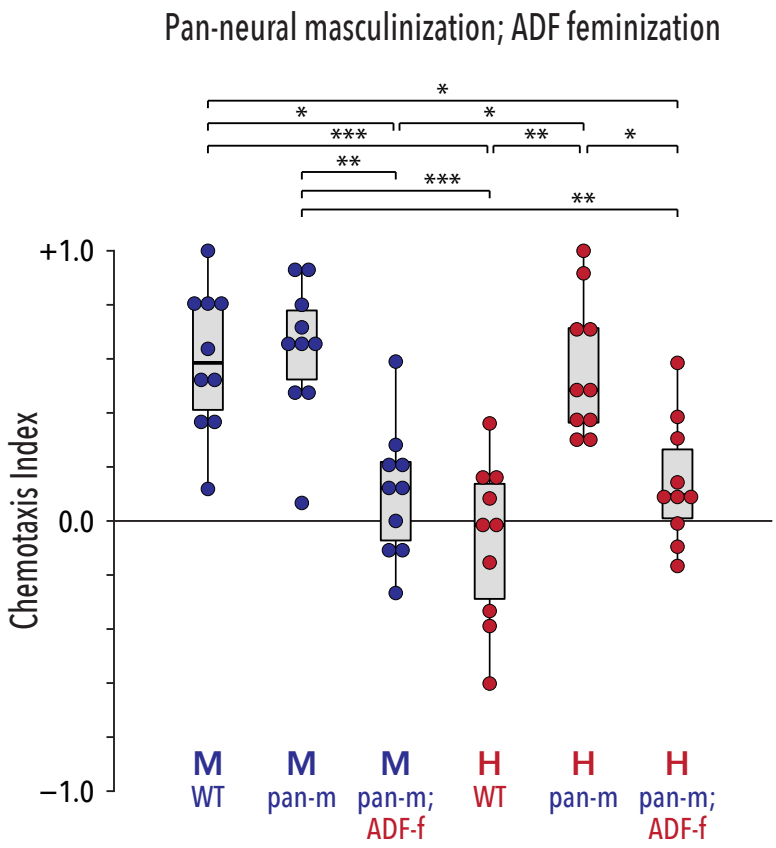

Figure S2. The genetic sex of ADF instructs behavioral valence, related to Figure 2.

The attraction to ascarosides generated by pan-neural masculinization in hermaphrodites ("pan-m") was suppressed by simultaneous feminization of ADF ("ADF-f"). In this case, the effects of feminization are expected to dominate, because expression of the feminizing transgene *tra-2(ic)* in ADF should block the effects of the masculinizing *fem-3(+)* transgene. In males, pan-neural masculinization had no apparent effect on behavior, but as expected, ADF feminization eliminated pheromone attraction. Groups were compared with Kruskal-Wallis analysis followed by Dunn tests with Holm's correction. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

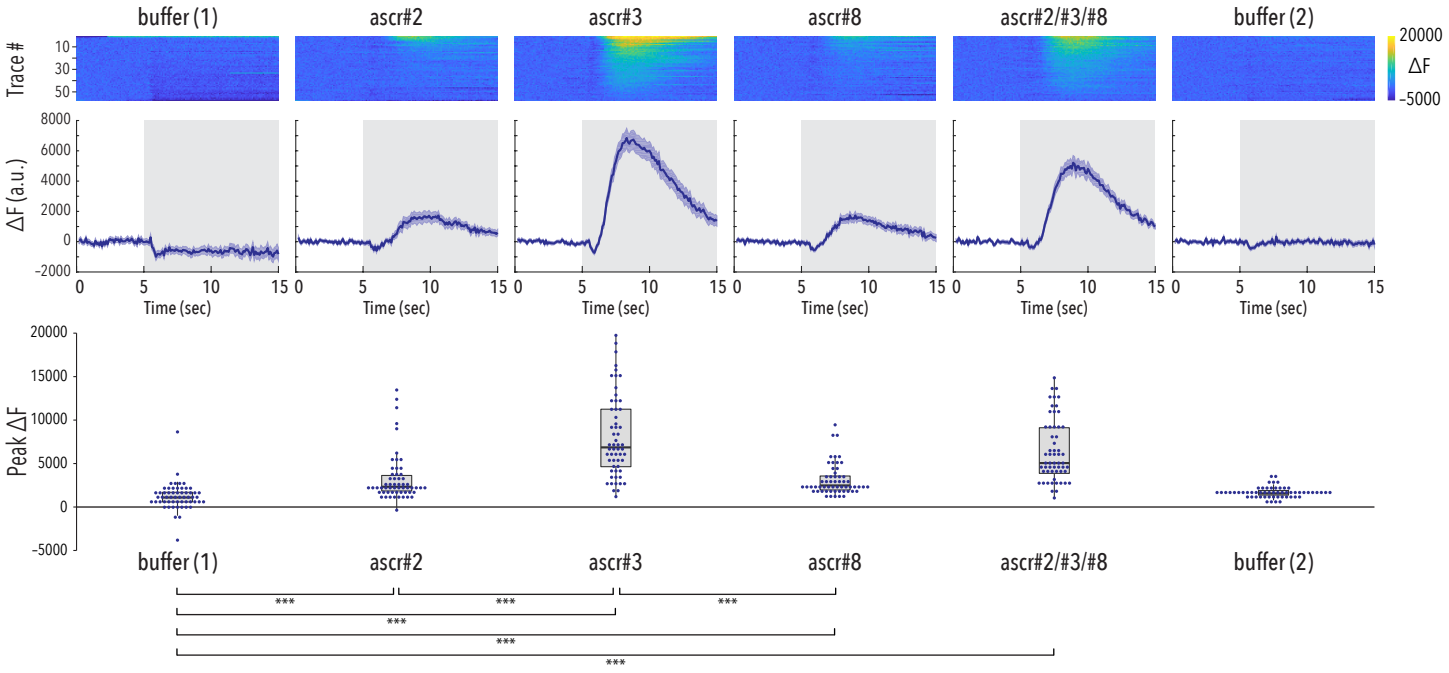

Figure S3. ADF responds primarily to ascr#3, related to Figure 3.

Upper panels show pseudocolored graphs of calcium recordings of individual neuronal responses to the indicated stimuli. Each animal underwent three rounds of stimulation; each row depicts a single response, sorted by peak activity. The pseudocolor scale represents GCaMP3 ΔF (blue lowest, yellow highest). *Middle panels* show average ΔF intensities ± SEM for the recordings depicted in the upper panels. Gray shading indicates the period of pulsed ascaroside stimulation. *Lower panels* show peak ΔF values for each recording; dots represent individual responses to each pulse. Stimulation with ascr#2 or ascr#8 alone elicited weak calcium responses, while stimulation with ascr#3 triggered a markedly stronger response. Because ascaroside stimuli were delivered successively to the same population of animals in the stimulus chamber, some adaptation may have occurred during the stimulation period. This limits the ability of strong quantitative conclusions to be drawn across compounds as compared to naive animals; however, the robust responses to the ascr#2/#3/#8 mixture at the end of each stimulus train strongly suggest that any adaptation that may have taken place is relatively minor. n=57 trials (19 animals, three pulses per stimulus per animal). Groupwise comparison of peak ΔF values was carried out using Kruskal-Wallis analysis and Dunn's posthoc test with Bonferroni correction. ***$p \leq 0.001$.

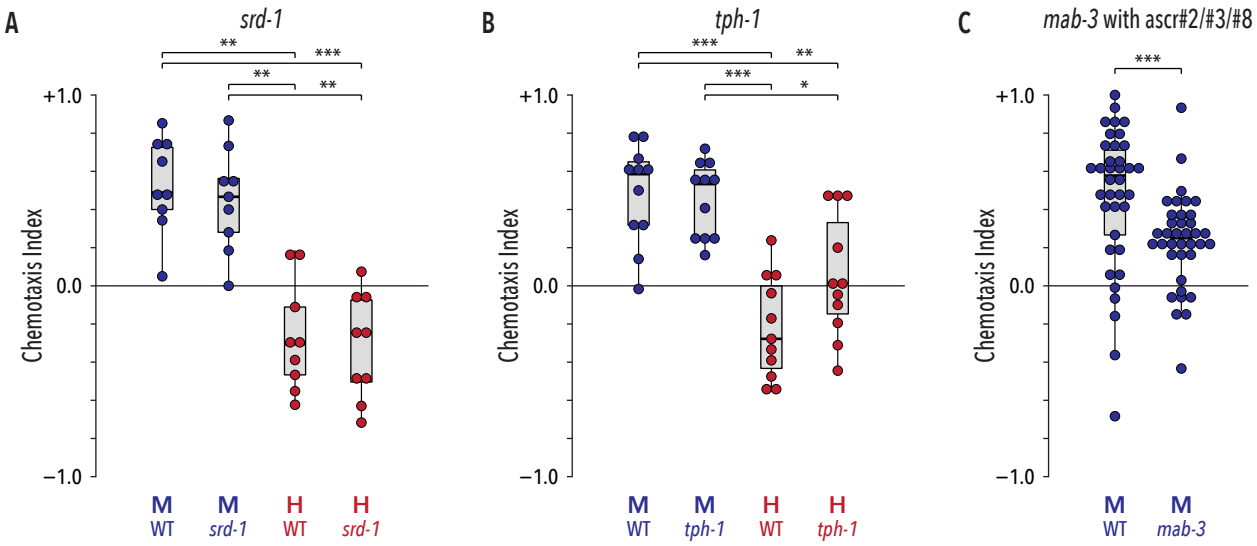

Figure S4. Genetic requirements for male pheromone attraction, related to Figure 4.

(A) The behavior of *srd-1* mutants in the quadrant assay (using the ascr#2/#3/#8 blend) was indistinguishable from wild-type. (B) Loss of the serotonin-biosynthetic enzyme *tph-1* had no detectable effect on the behavior of males or hermaphrodites using the ascr#2/#3/#8 blend. (C) *mab-3* mutant males exhibited a partial loss of attraction to the ascr#2/#3/#8 blend, compar able to the effects of *mab-3* loss on attraction to ascr#3 alone (see Figure 4A).

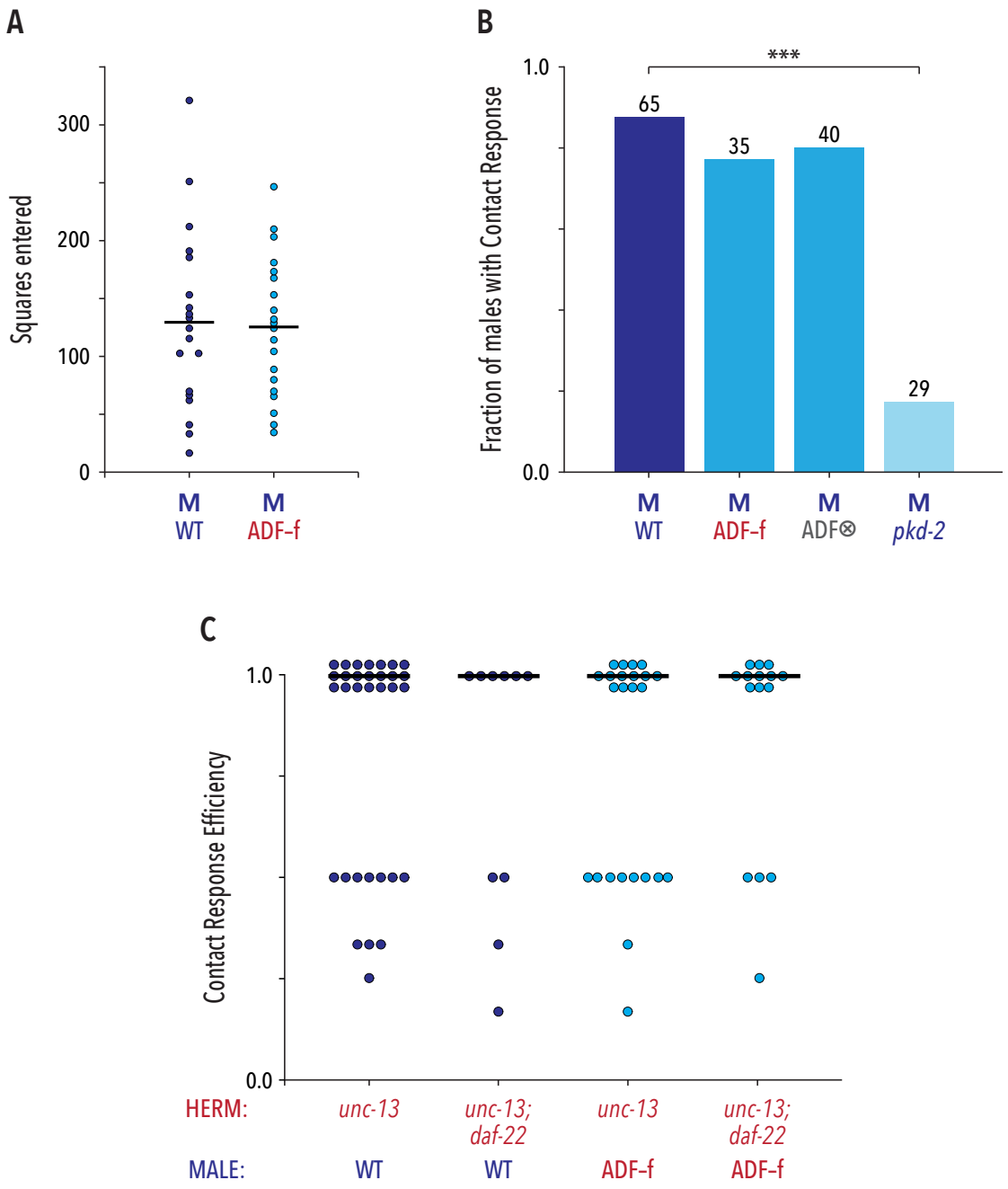

Figure S5. Feminization of ADF has no detectible effect on male exploratory behavior or the initiation of mating behavior, related to Figure 5.

(A) Male exploratory behavior, measured as the fraction of squares in a grid entered over 60 minutes, was not noticeably affected by ADF feminization. Each dot represents one male tested. Statistical analysis was performed using an unpaired Student's t-test. (B) Neither ADF feminization nor ADF ablation detectibly disrupted the frequency of contact response, the first

step of male mating behavior. *pkd-2* mutant males, whose response behavior is severely compromised [S1], were used as controls. Numbers of males assayed is shown above each bar. Statistical significance was assessed using Kruskal-Wallis analysis with Dunn's post-hoc tests. ***$p \leq 0.001$. (C) Contact response efficiency, scored as the reciprocal of the number of physical encounters until response occurred, was not detectibly affected by ADF feminization, nor did it depend on the ability of hermaphrodites to produce ascarosides (*unc-13* vs. *unc-13; daf-22*). Statistical analysis was performed with a Kruskal-Wallis test.

Supplemental References

S1. Barr, M.M., and Sternberg, P.W. (1999). A polycystic kidney-disease gene homologue required for male mating behaviour in *C. elegans*. Nature *401*, 386-389.